to characterise wild type and mutant proteins by polarographic and voltametric techniques to investigate the importance of these residues in ubiquinone-binding. Q242N, S256T and R262K substitution results in complete loss of AOX electron transfer activity that is not due to instability of the protein suggesting that these residues, also identified as being important in complex II, are critical for Q-binding in AOX. Of equal interest was the finding that N247Q substitution had little effect upon electron transfer or inhibitor sensitivity. We are currently investigating the importance of this residue since we believe it is responsible for a difference in sensitivity to ascofuranone, a very specific inhibitor of the alternative oxidase, in *Trypanosoma brucei* and *Trypanosoma vivax*. Results will also be presented as to the importance of Y253 and H262 in Q-binding.Supported by the BBSRC.

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S11.40 Over-expression, purification and crystallisation of the alternative oxidase

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The aim of this project is to elucidate the structure of the alternative oxidase under optimal crystallographic conditions. The alternative oxidase (AOX), an inner-mitochondrial membrane ubiquinol:oxygen oxidoreductase, is responsible for cyanide-resistant respiration in plants, several fungi and a variety of alpha-proteobacteria. Importantly, several human parasites, including *Trypanosoma brucei brucei* and *Blastocystis hominis*, also functionally express the alternative oxidase gene. In the case of trypanosomes, the causative agent of African sleeping sickness, AOX has been found to be the sole oxidase present in the bloodstream form of the kinetoplast parasite. While the alternative oxidase has been modeled *in silico*, the exact structure remains unknown. Detailed knowledge of the structure is essential to the future study of the enzyme, specifically in relation to rational drug design of effective anti-parasitic drugs. *Sauromatum guttatum* AOX has been over-expressed in both C41 and heme-deficient *Escherichia coli* strains, solubilised in the presence of a variety of detergents, and subsequently purified using cobalt affinity gel. Results will be presented to show the production of an active protein at all stages of the purification process, in addition to demonstrating for the first time that the plant AOX is sensitive to the specific trypanosomal inhibitor ascofuranone. This work is supported by a grant from the BBSRC.

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(S12) Mitochondria and disease symposium lecture abstracts

S12/1 Mitochondria function in the diabetic kidney

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The diabetic kidney has an altered energy metabolism which is partly due to the increased tubular electrolyte load, but also due to activation of several seemingly different pathways. We have previously shown that increased oxidative stress and activation of the polyol pathway result in reduced tissue oxygen tension throughout the diabetic kidney. Interestingly, we found that the reduced oxygen availability always was linked to increased oxygen utilization, even during situations of glomerular hypofiltration, i.e. when the tubular load of electrolytes was reduced.

We could show that the increased oxygen utilization in the diabetic kidney *in vivo* is dependent on reduced nitric oxide bioavailability, and thus reduced inhibition of mitochondria respiration, but exogenous stimulation of the nitric oxide production was not sufficient to alone normalize the oxygen tension. When investigating the cellular oxygen metabolism in the diabetic kidney at the molecular level, we found that the diabetic kidney has increased uncoupling protein (UCP)-2 expression. Oxygen consumption by diabetic mitochondria can be stimulated by glutamate alone, which is in vast contrast to mitochondria from normoglycemic controls. The glutamate-stimulated oxygen consumption by the diabetic mitochondria is prevented by either addition of GDP or removal of the free fatty acids, which further supports the conclusion of mitochondrial uncoupling. Although remaining to be supported by future experiments, we propose that the increased UCP-2 expression in the diabetic kidney is an antioxidant defense, which serves to reduce the mitochondria superoxide radical production and thereby preserve the function of the electron transport chain.

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S12/2 Pathogenic mutations in the mtDNA ATP6 gene and impairment of the ATP synthase energy transduction

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We used different human cells to elucidate the molecular mechanism responsible for biochemical and clinical phenotypes associated with mutations at nt8993 in the mitochondrial DNA (mtDNA). The most common human mutations at this nucleotide, T>G and T>C, change Leu-156 for Arg and Pro, respectively, in the ATPase6 subunit (homologous to subunit *a* of *E. coli*) of the F_1F_0-ATPase (ATP synthase). When Pro substitutes for Leu, both clinical and biochemical phenotypes are rather mild, and ATP synthesis rate is found less than 20% reduced, matching the decrease of proton translocation rate through F_0 during ATP synthesis. At variance, the mtDNA 8993T>G mutation, bringing in the ATPase6 subunit Arg-156 and being associated with severe syndromes of infancy and childhood, induces a dramatic decrease of ATP synthesis rate, an impaired proton translocation rate, but an almost normal ATP hydrolysis rate. Taking into account the above observations, and on the basis of structural prediction analysis of mutant ATPase6 subunit, we suggest that the proton translocation pathway through F_0 is impaired by the T>G mutation, possibly due to partial loss of the Leu-156-containing transmembrane helix, which is essential for energy transduction by the ATP synthase. In conclusion, our study demonstrates the important role of Leu-156 for the ATPase6 structure allowing mitochondrial F_1F_0-ATPase energy transduction, and it provides a molecular mechanism for the pathogenesis of severe human syndromes. Moreover, our results suggest that mitochondrial